A NOTE ON THE SPERMATOGENESIS OF TENEBRIO MOLITOR.

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This study was suggested to me by Dr. Nettie M. Stevens, to whom I am deeply indebted for direction and help during the earlier part of the work, carried on while fellow in biology at Bryn Mawr College in 1912.[1]

The form studied is the common meal-worm, *Tenebrio molitor*. The worms were kept in the laboratory in a glass jar, and the testes dissected out as needed in the progress of the work. Five methods of fixation were used: Flemming's strong solution, Hermann's platino-acetic-osmic preparation, Gilson's mercuro-nitrate, and Bouin's fluid. After Flemming or Hermann fixation the testes were stained with iron-hæmatoxylin, and with safranin followed by gentian-violet or lichtgrün; after Bouin, with iron-hæmatoxylin and with thionin; after mercuro-nitrate, with Auerbach's fuchsin-green, with thionin, and with iron-hæmatoxylin.

The safranin and lichtgrün staining after either Flemming or Hermann fixation gave the most satisfactory results. By this method the structures of the nucleus were more clearly differentiated than by any of the other staining methods. The iron-hæmatoxylin and the thionin after any of the fixing agents were next in favor, but they were both useless for some of the most important stages, as they stained linin and chromatin alike. Two of the iron-hæmatoxylin slides, however, were destained sufficiently to give a faint differentiation in that respect. Auerbach's stain was a complete failure with my material; and although Miss Stevens and I both tried the Benda method repeatedly, in no instance did we obtain a typical stain. In two slides of Miss Stevens's the differentiation of the mitochondria

[1] Owing to Miss Stevens's sudden illness and death the work has had to be completed without her supervision. It is only through the great kindness of Dr. T. H. Morgan and Dr. E. B. Wilson, of Columbia University, that I have been enabled to prepare it for publication. My many thanks are due them both for their help and direction.

was fair, though by no means typical, the mitochondria being stained violet, the chromosomes a deep purple.

In 1905 Miss Stevens investigated the spermatogenesis of *Tenebrio molitor* (05*a*), paying special attention to the *X* chromosome. Her only reference to the conjugation stage is one short sentence: "A brief 'synapsis' or condensation stage occurs at the close of the last spermatogonial mitosis." She gives one figure of this stage and one of a slightly later stage, both drawn from iron-hæmatoxylin material. The drawing of the condensation stage shows a dense mass of chromatin at one side of the cell. The other figure shows what I have called the "large-loop" stage.

These figures and the accompanying descriptions indicate that Miss Stevens at that time supposed the conjugation to be of the type described by her for three of the Coleoptera and two of the Lepidoptera ('06*a*) and for *Diabrotica vittata*, another of the Coleoptera ('08). The type as described for these forms was telosynaptic. The very short loops that appear in the synizesis stage, "a prolongation of the last spermatogonial telophase," later straighten out and unite end to end to form loops which at first very often have knobs or slight irregularities at the point where the two chromosomes have come together. These loops then pass directly over into the diffuse spireme.

The appearances in my material indicate a considerable divergence from this type.

In the spermatogonia of the meal-worm there are nineteen large chromosomes and one small chromosome, making twenty in all, as shown in metaphase in Fig. 1. Figs. 2, *a* and *b*, show two views of a spermatogonial telophase from a cyst surrounded on three sides by spermatocytes. I have inclined to believe this stage the last telophase preceding the maturation divisions.

The next succeeding stage that I was able to find is shown in Fig. 3; this cell came from a cyst in which every cell except the one figured showed a dense closely massed clump of chromatin at one side of the cell. This appearance is so usual and so well known I did not think it necessary to show a figure of it. It is very similar to the stages called variously "synizesis" and "condensation," and as in Miss Stevens's material, seems to follow directly on the last spermatogonial telophase. There may be

between these two stages such intermediate conditions as Dr. Wilson found in *Lygæus* and *Oncopeltus* ('12). The presence of such stages would probably change the interpretation I have put upon the stages described. I was unable, however, to find any such intermediate conditions, and so have been forced to hold to the present interpretation.

These contraction stages were very common in my material, but the section figured, which I have interpreted as a cross section of such a clump, is a rather rare occurrence. This is to be expected, since sections through many different planes would give a side view of a mass crowded at one side of a cell, while a section through one plane only would give such a polar view as is shown in Fig. 3.

I have interpreted this contraction phase as the stage at which conjugation takes place, thus placing synapsis much earlier in the process of spermatogenesis than Miss Stevens had done in her earlier work. According to this interpretation, in Fig. 3, chromosomes *a* and *b*, *c* and *d*, *e* and *f*, *g* and *h*, *x* and *y*, are cross sections of pairs of chromosomes not yet united; chromosomes *j* and *k*, *l* and *m*, *o* and *p*, are side views of such pairs; and *q* and *r* are the tops of the thickened loops formed by two joined chromosomes.

Miss Stevens followed the work up to this point, and it was under her direction that this stage came to be interpreted as the conjugation stage. It was her opinion that the type here was telosynaptic.

As the side view of this stage shows only a very dense, irregular chromatin mass, and the cross sections of this stage are so rare, the actual meeting of the two chromosomes would be very difficult to see; I did not succeed in finding it. But the fact that at the end of this stage never more than ten chromosomes can be found in a cell, while just before it (that is, in the last spermatogonial telophase) twenty were present, is in itself proof that conjugation takes place at this time. And the appearance of the short loops (Figs. 4, 5, 6, 7, 8) as the condensation stage opens up, is an indication of the manner in which synapsis has occurred.

The next stage in the observed process is the transformation of these short thick loops into long loops, partly linin, partly chro-

matin. This, I believe, has not been hitherto described for any form and is the phenomenon so effectively brought out by the safranin-licht-grün process of staining, the linin staining a clear green, the chromatin a deep rich red. By no other staining method that I used was this differentiation clearly brought out. In almost all preparations these loops appear to be homogeneous, as Miss Stevens has drawn them in her paper ('05*a*). Figs. 5, 6, 7, and 8 show several stages in this process of loop-formation. How this transformation is brought about I did not determine. At this stage there is usually some amount of green-staining material mixed in with the chromatin mass. Whether the linin of the loops comes from the chromatin or from this diffuse green-staining material I did not determine.

There are always ten or fewer chromosomes in sections of cells at this stage, which is the period of general growth. The chromatin at the tops of the loops thickens and becomes dumbbell-shaped, and both the cell and the nucleus nearly double in size (compare Figs. 8 and 13).

Toward the end of this period of general growth, the loops begin to straighten out and then to become attached to each other by their linin ends, thus forming the spireme (Figs. 9, 10 and 12). The spireme never becomes diffuse, but retains this appearance of dense chromatin masses, like dumbbells, strung upon a linin thread. This thread gradually becomes fainter and more slender, until at the stage just preceding the first spermatocyte division, it is seen as an almost indistinguishable double line connecting the chromosomes (Fig. 13). Rarely during these later stages can a chromosome be found in a tetrad form (Fig. 12).

Fig. 14 shows these ten bivalent chromosomes in the prophase of a first maturation division, which is the reductional division.

Conclusions.

1. No stages were found intervening between the last spermatogonial telophase and the contraction stage.

2. When the chromosomes have fully emerged from the contraction stage, they are in the form of loops, and are in the reduced number.

3. The loops formed by these bivalent chromosomes are not homogeneous, but are partly linin.

4. No diffuse "resting stage" was found, the chromatin being in the form of compact, definite bodies throughout the whole observed process.

5. No diffuse spireme was found.

LITERATURE.

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'06 Ibid., Part 2.

'08 The Chromosomes in *Diabrotica vittata*, *Diabrotica soror*, and *Diabrotica 12-punctata*. J. of Exper. Zoöl., Vol. 5, No. 4.

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Explanation of Plate I.

Fig. 1. Flemming; safranin and licht-grün. Spermatogonial metaphase, 20 chromosomes.

Figs. 2*a* and *b*. Ibid. Two views of a spermatogonial telophase.

Fig. 3. Flemming; safranin and licht-grün. Cross section of contraction stage; *a* and *b*, *c* and *d*, *e* and *f*, *g* and *h*, *x* and *y*, pairs of chromosomes in cross-section, not yet united; *j* and *k*, *l* and *m*, *o* and *p*, pairs of chromosomes in side view, ready to unite; *q* and *r*, tops of short loops formed by the thickening, shortening and thickening of two united chromosomes.

Fig. 4. Flemming, safranin and licht-grün. A little later stage in the contraction phase, showing loops.

Figs. 5, 6, 7, 8. Flemming, safranin and licht-grün. Formation of large loops.

Fig. 9. Flemming, iron-hæmatoxylin. Large loops beginning to straighten out.

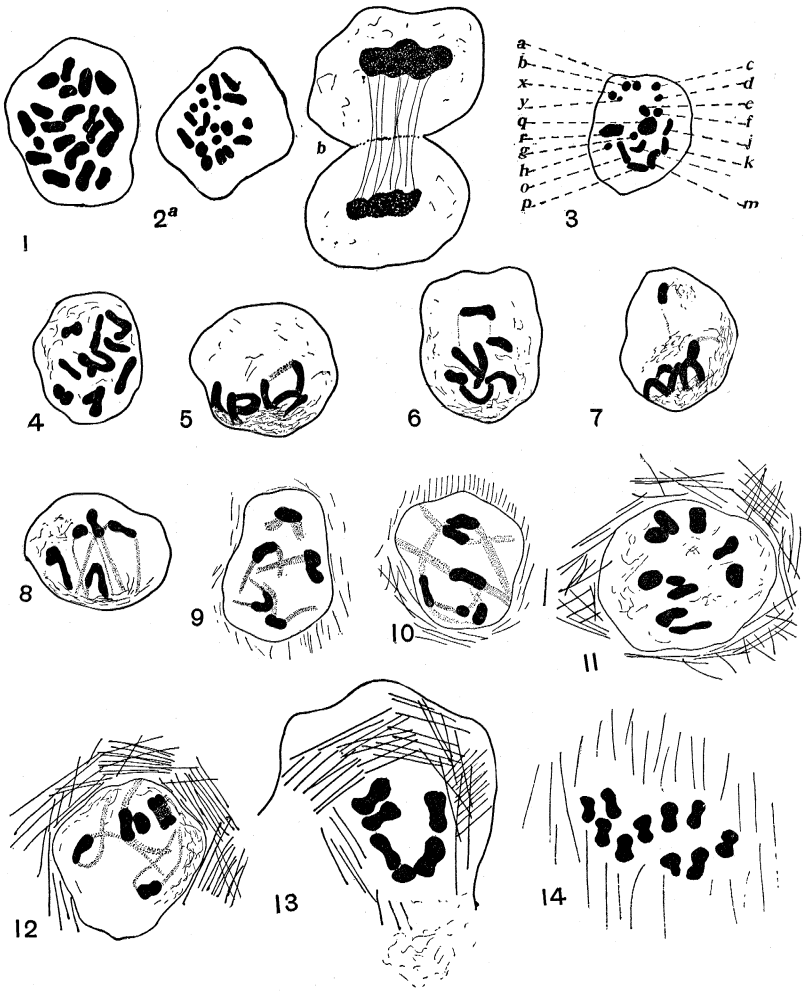
Fig. 10. Hermann, safranin and licht-grün. Formation of spireme.

Fig. 11. Hermann, safranin and licht-grün. Showing the ten chromosomes of this stage.

Fig. 12. Hermann, safranin and licht-grün. Spireme, showing tetrad.

Fig. 13. Flemming, safranin and licht-grün. Late spireme.

Fig. 14. Hermann, safranin and licht-grün. Prophase of first spermatocyte division, showing ten bivalent chromosomes, including the unequal pair.



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